



Cytotoxic Effect of Hydroalcoholic Extract from *Satureja bachtiarica* Bunge on Hela Cancer Cells Line

Anahita Behdarvand Shoushtar¹, Hossein Sazgar^{*1}, Abdollah Ghasemi Pirbaloti²

¹Department of Biology, Faculty of basic Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran;

*Email: hoseinsazgar@yahoo.com

²Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran;

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ABSTRACT

Background & Aim: Cervical cancer is the most common female genital cancer and the second most common cancer in women in the world. Since the herbal ingredients have been used for centuries to treat cancer, purpose of the present study was to determine the cytotoxic effect of hydroalcoholic extract from *Satureja bachtiarica* Bunge on Hela cancer cells line.

Experimental: Hela cells line and natural fibroblast cells line were cultured in RPMI 1640 and DMEM Containing fetal bovine serum and antibiotics, respectively. The cells were exposed to different doses of hydroalcoholic extract of *Satureja bachtiarica* Bunge (0.156, 0.312, 0.625, 1.25, and 2.5 mg/ml) and were incubated for 24, 48 and 72 hr, respectively. After the incubation period, the modified MTT colorimetric test was used in order to determine cell toxicity of the extract.

Results: The results of MTT test Showed that hydroalcoholic extract of *Satureja bachtiarica* Bunge has dose-dependent anti-cancer effect on Hela cancer cells, so that by increasing the concentration of extract and incubation in 72h, the most percentage of cell death was observed ($p < 0.05$). The extract of plant didn't show any significant cytotoxicity on natural fibroblast cell line. Therefore, it seems that its compounds will be applied in treating cancer through more researches.

Recommended applications/industries: Considering the low side effects of herbal medicines, especially antioxidants including *Satureja bachtiarica* Bunge can be used to treat cancer with drug therapy.

1. Introduction

Cancer is one of the most common causes of death in human societies and according to the World Health Organization report, it is involved in 13% of all deaths across the world. Cervical cancer is the sixth most common cancer among any types of cancer

(Mortazavian *et al.*, 2012), cervical cancer is the most common cancer of the female reproductive system (Vaisy *et al.*, 2013) and it is considered as the second leading cause of cancer death in women (Giusepe *et al.*, 2008), which most of these people live in developing countries (Castellsague *et al.*, 2011).

Cancer treatment is done with surgery, chemotherapy, hormone therapy, radiation therapy,

immunotherapy. Often cause various side effects that chemical treatment (DeVita *et al.*, 2012). Because many chemical drugs cause digestive disorders, kidney damage and so forth, scientists attempt to find drugs which have less side effects than chemical drugs, in this regard medicinal plants have been considered. Due to having other compounds along with special pharmacological effects, medical plants have fewer side effects than chemical drugs (Forouzandeh *et al.*, 2014). Many plants and herbs contain anti-cancer elements whose effects can enforce in different stages of growth of cancer cells (Abdullaev, 2001). The main aim of cancer prevention through chemical or natural products is to slow or inhibit carcinogenic process. This approach focuses on the abnormal intracellular pathways that lead to abnormal cell functions (Aggarwal *et al.*, 2007).

Now it is accepted that the plants, vegetables, spices and traditional medicines which are used among the people can act as a key resource for cancer prevention (Abdullaev, 2001). Food factors play important role in preventing cancer. Countless numbers of patients around the world use medicinal plants in order to maintain their health. So, scientists have deeper look at the biology properties, therapy power and health of these products (Abdullaev *et al.*, 2001).

Satureja is from the genus of the mint family Lamiaceae which about 14 species of it have been reported in Iran. *Satureja bachtiarica* with the scientific name *Satureja bachtiarica* Bunge has relatively wide dispersion in Iran and grows in various parts of the country such as Chahar Mahal and Bakhtiari, Lorestan, Khuzestan, Ilam, Kermanshah, Isfahan, Gilan and some other places. This species has leaves that fold over on the length and have linear rectangular form which are placed along the stem as integrated (Sefidkon *et al.*, 2007). *Satureja bachtiarica* Bunge has relatively hot and dry nature, anti-bloat and appetizer and is effective in reinforcing sexual power. It is also used for dental pain and if is eaten with fig juice, it has a beneficial effect for cough and dyspnea (Sefidkon and Jamzad, 2005).

In the studies on *Satureja bachtiarica* Bunge, it was shown that tannins, fatty substances such as terpenoids and phenolics compounds are the main effective and bioactive components in its extracts, as well as the recent studies on the *Satureja bachtiarica* Bunge identified that Paracemen, carvacrol and thymol compounds are the most important constituents of the plant (Dadfar *et al.*, 2013).

Cell culture is one of the novel methods of study and investigation and its traces can be found in almost all scientific disciplines. One of the goals of cell culture is to study the cells in term of grow, food requirements and reasons of their grow stop. So in order to study the cell cycle, development of controlling the growth of cancer cells and modulating gene expression, these cells should be cultured in vitro (Forouzandeh *et al.*, 2014).

Hela cells are a class of human cancer cells that were isolated from cervical cancer in 1951 and now are used in many studies on cancer cells (Scherer *et al.*, 1953). The present study was done aiming at examining the anti-cancer effect of hydroalcoholic extract of *Satureja bachtiarica* Bunge on cervical cancer cells (Hela).

2. Materials and Methods

2.1. Extraction

Aerial parts including stem and leaves of *Satureja bachtiarica* Bunge were collected from Chahrmahal va Bakhtiari province and rotary method was used for collecting and extracting its hydroalcoholic extract. For extraction, the leaves and stems of plant were dried in the shadow, powdered by mechanical mill, then the powder was poured into cylinder and solvent was poured on it. The used solvent was ethanol 90% which had been mixed with water. This hydroalcoholic solvent was used to the extent that completely covers the plant powder. The resulting solution was then placed in the oven that was set to 50 ° C. After 72 hr in the oven, solution was exited from the machine and passed through the filter paper. Then the filtered solution was placed in stearic rotary machinery in several steps so that it becomes concentrated. The resulted extract was used to prepare the extract in different doses.

2.2. Cell culture

Hela cell line of cervical cancer and fibroblast cell line of natural cell were provided from National Center for Genetic Resources of Iran. For culturing Hela cells, cultivation environment RPMI 1640 (Roswell Park Memorial Institute 1640) and for culturing fibroblast cells, cultivation medium DMEM (Dulbecco's Modified Eagle Medium) along with FBS (Fetal bovin serum) 10% and 1% Penicillin-Streptomycin was used and they were cultured under incubator standard conditions (temperature of 37c ° And 5% Co₂ And humidity 95%). After three passages, cells were used for later

processing. Cell counting and number of live cells was done by Trypan blue.

2.3. MTT (Methyl Tetrazolium) test

In order to measure the cytotoxicity of hydroalcoholic extract of *Satureja bachtiarica* Bunge, MTT test was used. In this method, Salt Methyl thiazolyl tetrazolium bromide or MTT which is yellow was transformed to insoluble purple formazan through mitochondrial dehydrogenase enzymes of active cells, light absorbance of this compound is measurable after dissolving in DMSO (dimethyl sulfoxide) by using Eliza reader and in the 429-630 nm wavelength (Galati and Brien, 2004).

2.4. Toxicity of *S. bachtiarica* extract by using MTT test

After covering the flask bed with cell, cell layer sticky to flask bottom was separated in enzyme method and by using Trypsin and was centrifuged in 1200 rpm in 5 min after transferring to sterile test tubes. Then cells were suspended by using Pasteur pipette in new culture medium and cell suspension was provided from them. After counting, cells were poured in smooth-floor 96-well plates as 10^4 cells and plates were incubated at 37 °C for 24 hours. After the required time, the supernatant was removed slowly and carefully and new medium and hydroalcoholic extract of *S. bachtiarica* Bunge in concentrations 0.156, 0.312, 0.625, 1.25 and 2.5 mg/ml were added to all wells. Serum containing medium without extract was added to control wells. Plates were incubated for 24, 48 and 72 h. After the incubation period, plates were removed from the incubator, supernatant of each well was completely removed by sampler, cells were washed with 100 ml PBS (Phosphate-buffered saline) and then 80 microliter medium and 20 ml yellow MTT solution was added and the plates were incubated for 3 hours, after the required time, first the supernatant was completely removed and each well was washed with 100 ml PBS and 100 ml DMSO was added to dissolve formazan crystals, then the resulted color change was read by device Eliza reader at a wavelength of 492-630 nm.

In order to convert the amount of light absorption (OD) the percentage of live cells, the following formula was used and life percent of cells after 24, 48 and 72 h was computed.

Biological ability percent = $OD\ Control / OD\ Test \times 100$

A concentration of tested compound which halved the cell viability was considered as IC₅₀ (The half maximal inhibitory concentration).

2.5. Statistics methods

The data was analyzed by appropriate statistical methods in Excel and by using ANOVA and Tukey methods. Differences between the various groups was significant in $p < 0.05$.

3. Results and discussion

3.1. Effect of extract of *S. bachtiarica* on bio-viability of *Hela* cancer cells

Statistical analysis of results showed that during the 24-hour incubation, cell viability was increased by increasing the doses of the hydroalcoholic extract of *S. bachtiarica* Bunge in *Hela* cell line. So, that viability percent was decreased from 78.2% in concentration 0.156 mg/ml to 16.9% in 2.5 mg/ml, and the difference was statistically significant ($p < 0.05$).

In 48-hour incubation, dose-dependent reduction of viability was observed. So that the percentage of viability was decreased from 74.83% in 0.156 mg/ml concentration to 12.09% in concentration 2.5 mg/ml, it was statistically significant ($p < 0.05$).

In 72-hour incubation, the reduction of viability percent was also observed from 64.36 % in concentration 0.156 mg/ml to 10.69% in 2.5 mg/ml, it was statistically significant ($p < 0.05$).

Statistical analysis by using ANOVA test and Tukey showed significant difference at all concentration and in all three time in this cell line ($p < 0.05$). The most toxicity effect was observed in concentration 2.5 mg/ml and 72 h incubation (Figure 1). 50% cell growth inhibitory concentration (IC₅₀) hydroalcoholic extract of *Satureja bachtiarica* Bunge was obtained for *Hela* cancer cells as 0.312 mg/ml.

3.2. Effect of *S. bachtiarica* extract on bio-viability of natural fibroblast cells

Natural fibroblast cells with various concentrations of hydroalcoholic extract of *Satureja bachtiarica* Bunge were treated for for 24, 48 and 72 hours. The results of MTT test indicate that hydroalcoholic extract of *Satureja bachtiarica* Bunge didn't have any significant effect on natural fibroblast cells (Figure 2).

Hela cancer cell growth after 72 hours of treatment with hydroalcoholic extract of *S. bachtiarica* Bunge at a concentration of 2.5 mg/ml (Figure 3.B) has been inhibited in comparison to the control group (no treatment with hydroalcoholic extract of *S. bachtiarica* Bunge), (Figure 3.A). After exposure to a concentration of 2.5 mg/ml of hydroalcoholic extract of *S. bachtiarica* Bunge, cells were distorted and their morphology was changed, showing toxicity effect of hydroalcoholic extract of *S. bachtiarica* Bunge on these cells.

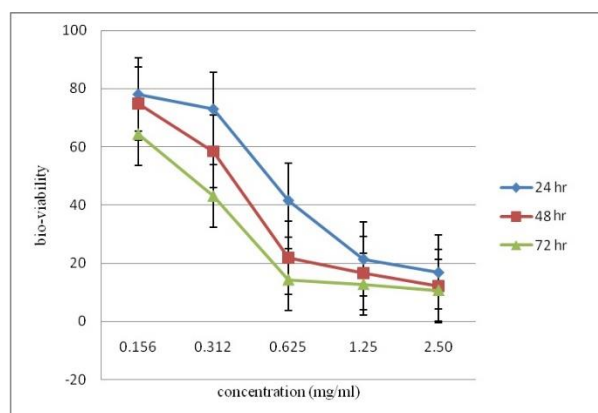


Fig. 1. The changes in bio-viability of hydroalcoholic extract of *Satureja bachtiarica* Bunge on Hela cancer cells in different concentrations and times by using MTT method.

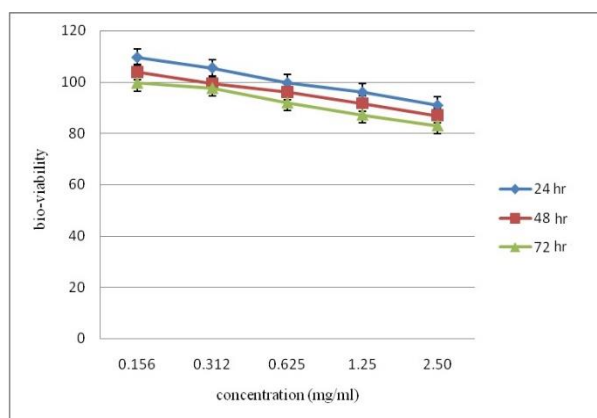


Figure 2. The changes in viability of hydroalcoholic extract of *Satureja bachtiarica* Bunge on natural fibroblast cells in different concentrations and times by using MTT.

In the present study, the toxic effects of hydroalcoholic extract of *S. bachtiarica* Bunge on Hela cancer cells line and natural fibroblast cells were

studied. The results show that hydroalcoholic extract of *S. bachtiarica* Bunge has cytotoxic effects on Hela cancer cells, while it doesn't have any toxic effect on natural fibroblast cells.

Many herbs and spices have pharmacological and biochemical properties including antioxidant and anti-inflammatory ones, so that it appears that they involve in anti-neoplastic activity and cell mutagenicity (Shukla and Singh, 2007).

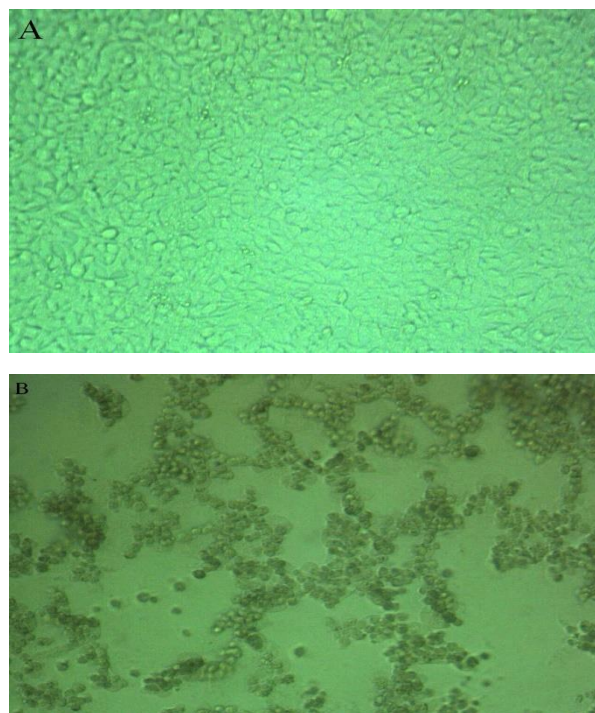


Figure 3. (A) Hela cancer cells in the control group without treatment with hydroalcoholic extract of *S. bachtiarica* Bunge (B) Hela cancer cells after 72 hour treatment with hydroalcoholic extract of *S. bachtiarica* Bunge at a concentration of 2.5 mg/ml ($\times 10$).

S. bachtiarica has much antioxidant property probably due to presence of phenolic compounds such carvacrol and thymol in this plant (Buchanan and Shepherd, 1981). Sefidkon and Jamzad (2004), stated this plant can be considered as a source of antioxidant.

Phenolic compounds have many biological properties such as antioxidant, trapping free radicals and anti-inflammatory properties, it also prevents or delays oxidative damages in lipids and other important molecules (Kris-Etherton *et al.*, 2002), as development of cancer is closely related with inflammation and

oxidative stress, antioxidant and anti-inflammatory compounds can be an anti-cell carcinoma (Moheghi *et al.*, 2011).

Studies have shown that plant extracts rich in phenolic compound lead to cell protective effects by reducing oxidative stress. Phenolic compounds are aromatic secondary metabolic plant categories which have a strong potential to wipe out free radicals widely distributed throughout the plant. Antioxidant activity of phenolic compounds in plants is mainly resulted from their regeneration power and chemical structure, allowing them to neutralize free radicals, from complexes with metal ions and turn off the single and triple oxygen molecules. Phenolic compounds inhibit oxidation reactions through donating electron to free radicals (Pokorny, 2007; Kumaran and Karunakaran, 2006; Wu *et al.*, 2006). So it is possible that phenolic compounds such as thymol and carvacrol in hydroalcoholic extract of *S. bachtiarica* Bunge reduce oxidative stress through inhibiting free radicals.

Angel *et al.* (2009) considered antioxidants compounds such as phenolic compounds as the effective factors on cytotoxic capacity *Artemisia Campesteris*, phenolic compounds protect cells against reactive oxygen species (ROS).

Health and useful effects of the plants can be attributed in part to the presence of phenolic substances. Many studies have been done in this field, including studies by Jamshidi *et al* (2010) have shown that there is an appropriate relationship between antioxidant activity and phenolic compounds of the plants. For example, mint and rosemary extracts have antioxidant activity and this activity is directly related to the phenolic content of the plant.

Thymol and carvacrol are among the major components of *S. bachtiarica* (Buchanan and Shepherd, 1981) to which various biological effects can be attributed. Studies have shown that these compounds play significant role in reducing oxidative stress and cyclooxygenase enzymes. On the other hand, various investigations show that cyclooxygenase enzymes play major role in carcinogenic mechanism (Keramati *et al.*, 2011).

Diaz-Cruz *et al.* (2005) found that cyclooxygenase through producing Prostaglandin E2 lead to increasing aromatase enzyme which can convert androgen to estrogen. Since estrogen promotes tumor growth; however, possibly with aromatase inhibition by

cyclooxygenase inhibitors, estrogen levels decrease and tumor growth becomes slow (Keramati *et al.*, 2012). So it is likely that phenolic compounds such as thymol and carvacrol in hydroalcoholic extract of *S. bachtiarica* Bunge result in reducing aromatase enzyme and subsequently estrogen and finally less tumor growth.

Therapeutic and preventive effects of ethanol extract of *Thymus vulgaris* on precancerous lesions and carcinoma of the prostate gland cells of Wistar rats were seen which were related to thymol and carvacrol (Singh and Lucci, 2002). Therefore, in this study it can also be said that thymol and carvacrol of hydroalcoholic extract of *S. bachtiarica* Bunge increase the cytotoxicity effect of the extract.

In the study by Hamta and Ghazaghi (2004), effective compounds of hydroalcoholic extract of *Thymus vulgaris* were responsible for apoptosis induction in cancer cells 4T1 and anti-cancer and cytotoxic properties of ethanol extract of thymol can be attributed to such compounds as thymol and carvacrol. Probably in this study, cytotoxic effect of hydroalcoholic extract of *Satureja bachtiarica* Bunge can be attributed to phenolic compounds such as thymol and carvacrol, consistent with the results of peer research.

The results of this study suggest that dose- and time-dependent hydroalcoholic extract of *S. bachtiarica* Bunge results in inhibiting the growth of Hela cancer cells, so that by increasing the duration and dose, cancer cell growth was inhibited and a significant difference was seen among three treatment times in 24, 48, and 72 hr.

Findings of the present investigation are consistent with those of Foroozande *et al.* (2014a), who studied anti-cancer effect of *Peganum harmala* L hydroalcoholic extract on Hela cancer cells. They showed that *Peganum harmala* L hydroalcoholic extract with dose-dependent and time-dependent effect on cancer cells can inhibit the growth of these cells.

Foroozande *et al.* (2014b) also studied anti-cancer effect of *Boswellia Serrata* hydroalcoholic extract on Hela cancer cells. They showed that *Boswellia Serrata* hydroalcoholic extract with dose-dependent and time-dependent effect on cancer cells can inhibit the growth of these cells.

The results of this study open a bed for further studies on anti-cancer effect of different types of medicinal herbs, so it is recommended that future studies

will be done in order to identify other active ingredients in a variety of medicinal plants to take advantage of clinical and practical using of these natural and available products.

4. Conclusion

With an overall look at the findings of this study, it can be said that hydroalcoholic extract of *Satureja bachtiarica* Bunge has anti-cancer effect which can inhibit the growth these cells through dose-dependent and time-dependent effect on Hela cancer cells. So that as time spends and in higher doses, growth of cancer cells was more inhibited. hydroalcoholic extract of *S. bachtiarica* Bunge did not have any significant effect on natural fibroblast cells.

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